From the
INTERNATIONAL SEARCHING AUTHORITY

To:
RONALD W. CITKOWSKI
GIFFORD, KRASS, GROH, SPRINKLE
ANDERSON & CITKOWSKI, P.C.
280 N. OLD WOODWARD AVE., STE. 400
BIRMINGHAM, MI 48009

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*)	16 FEB 2005
Applicant's or agent's file reference NEN-22352/16	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US04/18524	10 June 2004 (10.06.2004)	12 June 2003 (12.06.2003)

International Patent Classification (IPC) or both national classification and IPC

IPC(7): 33/533;33/53 and US Cl.: 436/546;435/7.72

Applicant

MARK BOBROW

1. This opinion contains indications relating to the following items:

- [x] Box No. I — Basis of the opinion
- [ ] Box No. II — Priority
- [ ] Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV — Lack of unity of invention
- [x] Box No. V — Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI — Certain documents cited
- [ ] Box No. VII — Certain defects in the international application
- [ ] Box No. VIII — Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(*b*) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/ US	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Shafiqul Haq Telephone No. 703-308-0198

Form PCT/ISA/237 (cover sheet) (January 2004)

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material

      ☐ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material

      ☐ in written format

      ☐ in computer readable form

   c. time of filing/furnishing

      ☐ contained in international application as filed.

      ☐ filed together with the international application in computer readable form.

      ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. V Reasoned statement under Rule 43 *bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims NONE	YES
	Claims 15-18 and 20	NO
Inventive step (IS)	Claims NONE	YES
	Claims 1-18 and 20	NO
Industrial applicability (IA)	Claims 1-20	YES
	Claims NONE	NO

2. Citations and explanations:

a) Claims 15-18 and 20 lack novelty under PCT Article 33(2) as being anticipated by Tsai et al. Tsai describes synthesis of activity probes for glycosidases (see abstract) consisting of a recognition head (Glucose) connected through a beta-glycosidic linkage to a p-hydroxy benzylic fluoride moiety which is connected to a reporter (biotin) through a lnker. The product of the reference meets all the limitations and anticipates the product of the instant claims 15-18 and 20.

b) Claims 1-14 lack an inventive step under PCT Article 33(3) as being obvious over Tsai. Tsai is applied for the reasons stated in paragraph a) above. In view of the fact that the Tsai et al. compounds are known to be useful as activity probes for a variety of applications (page 3609, last paragraph), it would be obvious to use these compounds in other well known conventional assay formats, as claimed. The packaging of reagent components in kit form (claim 14) is a well known expedient for ease and convenience in assay performance.

c) Claim 1-20 lack an inventive step under PCT Article 33(3) as being obvious over (1) Chen et al. in view of each of (2) Zhu et al., Lo et al. and Tsai et al. Chen describes a strategy for activity based detection of enzymes in a protein microarray. In the strategy described by Chen, immobilized proteins on microarray slides are allowed to react with cyanine labeled conjugate containing recognition head (eg phenyl phosphate group) that is connected to a trapping device derived from p-hydroxymandelic acid, which takes advantage of the quinine methide chemistry. Phosphatases enzymes present in the array remove phosphate group which leads to reactive quinine methide intermediate that in turn alkylate nearby nucleophiles on the biocatalyst resulting in the cyanine enzyme which can be detected by fluorescence detector. Besides cyanine, use of other type of equivalent reporters (eg. biotin, dansyl) is disclosed by Lo et al.

Claims 1-20 lack an inventive step under PCT Article 33(3) as being obvious over the prior art as applied in the immediately preceding paragraph and further in view of Tsai et al. Zhu et al. and Lo et al. used phosphate recognition head for detection/inhibition of phosphatase enzymes but Tsai et al. disclosed detection of other enzymes (eg. glycosidases) by using beta-glycosidic linkage at the recognition head (See abstract) and suggests using various sugar units and different aromatic configurations in combination with different linker/reporter to use for various applications and needs (page 3609, column 2, lines 19-37).
Therefore, considering the above fact, it would have been obvious at the time of the invention to a person of ordinary skill in the art to use/modify the strategy of Chen and use conjugates with different head groups and/or different reporters (eg cyanine, BODIPY, biotin, dansyl) as taught by others (Zhu et al., Lo et al. and Tsai et al) to detect different analytes (eg. enzymes) for various applications. The packaging of components in kit form (claims 14) is a well-known obvious expedient for ease and convenience in assay performance.

d) Claims 1-20 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.